

INSTITUT PASTEUR
CELL BIOLOGY AND INFECTION DEPARTMENT
DIRECTOR : Pascale COSSART
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The Cell Biology and Infection Department: concept /history/ objectives

Our department was created in 2002 by a group of pasteurians convinced that the study of the biology of the eukaryotic cell for itself or during host-pathogen interactions is absolutely necessary to fully understand patho-physiological processes, and that the Institut Pasteur would greatly benefit by having a department comprising both microbiologists and cell biologists.

Our objectives were :

1. To develop/ boost the study of the interface between microorganisms and cells – i.e. the growing discipline of Cellular Microbiology- whatever the nature of the micro-organism (bacteria, parasites, viruses or other infectious agents, e.g. prions).
3. To re-stimulate studies on the mammalian cell itself, i.e. fundamental Cell Biology, at the Institut Pasteur following the departure of several important cell biologists in the mid-nineties.
2. To create a multidisciplinary department hoping that important discoveries would be made at the frontiers between disciplines.
4. To initiate and establish collaborations with other departments in particular those of Immunology and Developmental biology, with Cell Biology serving as a common language to facilitate interactions

The department was thus created in 2002 by already well established groups : four « Pathogenesis » groups (Cossart, Guillen, Philpott, Sansonetti), three « Cell Biology » groups (Dejean, Israel, Nehrbass), three groups focusing on both Cell Biology and Cellular Microbiology (Dautry, Kean, Kellerman and Zurzolo who arrived in 2003) and three groups focusing on cell imaging : two platforms (Prevost and Shorte) and an image analysis unit (Olivo-Marin).

In 2009, the basic core composition of the department has not significantly changed (see Table 1). Yet a real evolution has occurred : Dana Philpott (a G5 group) has left the Institute. Kathie Kean's unit was closed. The unit directed by Ulf Nehrbass, now the Director of the Institut Pasteur in Korea, was recently closed. Three G5 groups have been created : those of Sandrine Etienne Manneville (created in 2005), Jost Enniga and Christophe Zimmer (these two latter were both created in 2008).

The Cell Biology and Infection Department: Overview

The Department in 2009 regroups 9 units, 3 G5 groups, and two platforms with a total personal of about 160 people investigating the mammalian cell itself and/or host-pathogens interactions at various levels with a special emphasis on the cell biology aspects and a strong interest in visualizing the various events under study.

Such a Department is a rather unique strength, not found in any other academic or private research institution. Our major challenge is to remain at the cutting edge of both Cell Biology and Infection Biology.

Research in the department aims at understanding the molecular mechanisms underlying either the different steps of an infectious process, or the activation of a particular cellular signaling cascade(Notch, NF- κ B), or the key signals or deregulations leading to a particular cellular phenomenon(endocytosis, migration, polarisation, cancer, senescence). Pathogens under study are diverse: we study both model pathogens, i.e. pathogens studied since

decades for which our knowledge is quite strong (*Shigella*, *Listeria*...) and also pathogens more difficult to address either because of lack of genetic tools (*Chlamydia*), of convenient growth culture conditions (*Entamoeba histolytica*) or simply lack of basic knowledge (prions) but which are of particularly relevant medical importance...

We not only focus on the successive steps of the cell infectious process - among them, entry, intracellular motility, cell to cell spread... -, we also study *in vivo* the behaviour and dissemination of pathogens within the host with a particular interest on the pathogen interaction with and the crossing of host barriers. Intense efforts have focused and still focus on the interactions of pathogens with the intestinal barrier but other tissues and organs (liver, lungs) are also under study. With the increasing availability of genomic data and high throughput technologies (affymetrix whole genome arrays, miRNA arrays, deep sequencing ...), we are particularly interested in investigating the specific host responses to pathogens, at the cell level, at the tissue level and also at the whole organism level. Analysis at the single cell level will be one of the most challenging issue for the future.

We also devote intense efforts to understand the mechanisms and identify the specific effectors used by pathogens to interact with and escape from the innate immune response or to dampen the innate immune response. We are also actively interested to understand the onset of the adaptative immune response.

Efforts in dissecting and understanding fundamental cell biology processes are mainly based on experimental systems but also in some cases on clinical data.

Most fundamental cell biological processes under study in the department have relevance for the study of the infection, for example the study of NF- κ B, the understanding of cell polarisation, the analysis of endocytosis.... In all cases, we try to visualize the events by the most performant imaging techniques taking advantage of the highly performant technical platforms available in the Institut Pasteur and of which two are part of our department. In fact, we contribute to the constant improvement of the platforms by our requests and also our interest in their improvement. The department is highly interested in automated and quantitative image analysis and we have now two groups whose main focus is the exploitation of image data. Several important internal collaborations with these two groups within the department and also outside the department have led to important breakthroughs.

During the last few years, the department has been highly productive with important results published in the highest impact journals such as Nature, Science, Cell, Cell Host and Microbe, Development Cell, Molecular Cell, Nature Immunology, Nature Cell Biology, Nature Methods, Journal of Experimental Medicine, PNAS, PLoS Pathogens, Journal of Cell Biology, Current Biology, Embo Journal and other internationally recognized journals of speciality, Cellular Microbiology, Molecular Microbiology, Traffic, IEEE Transactions Image Processing, Applied optics...

The department has led to the emergence of three G5 in 2008 (Enniga, Lecuit, and Zimmer).

The department has an active scientific life with regular and very well attended seminars. We organize a departmental retreat every year, either within the Institut Pasteur or outside (Dourdan, Le Croisic...). In addition, G5 group leaders present their work to the other group leaders once a year.

The department is highly involved in teaching. It is responsible (in collaboration with the Curie Institute) for the Course of Molecular Biology of the Cell (main organizers Chiara Zurzolo and Philippe Chavrier (Institut Curie)). It is responsible for the Course of Microbiology (main organizers Olivier Dussurget from the Department of Cell Biology and Infection with Chantal LeBouguenec from the Department of Microbiology). It is also responsible for the

Course of Vaccinology (Armelle Phalipon). Important links with the University (Cell biology of parasites course organized by Nancy Guillen) and the Collège de France (Philippe Sansonetti) have been established. S. Etienne-Manneville is Professor at the Ecole Polytechnique.

The department has organized (main organizers: Javier Pizarro-Cerda, Guy Tran Van Nhieu and Pascale Cossart) in 2008 an Embo practical course entitled « Host Pathogen interactions ».

The department (and in particular P. Sansonetti with P. Cossart) has been at the origin of the « Rencontres des Ravatys » between the CIML (Centre d'Immunologie de Marseille Luminy) and the two departments of Cell Biology and Infection and of Immunology of the Pasteur Institute. This meeting in Le château des Ravatys which took place in 2007 and 2008 will again take place in 2009. This meeting is aimed at stimulating interactions and collaborations between microbiologists and immunologists.

Interactions with European colleagues take place through several European programs, including the Network of excellence Pathogenomics, and « EIMID » (European Initiative for basic research in Microbiology and Infection Diseases). EIMID as well as the INTRAPATH program have brought to the department several European PhD students .

The department has organized in the Institut Pasteur the first Institut Pasteur Institut Curie meeting in January 2009. A second meeting will take place in Institut Curie next year.

The department is part of the Institut Carnot Pasteur Maladies Infectieuses which is aimed at stimulating translational research towards industry. The director of the Institut Carnot Pasteur is one of the Unit heads of the department (JC Olivo-Marin). A common project on sepsis which will involve many groups of the department is at present submitted for funding to the Institut Carnot Pasteur.

Our department is attracting a lot of internal interest since six groups from different departments of the Institut Pasteur have requested to have a secondary affiliation to our department (Andres Alcover from the Immunology Department, Carla Saleh from the Virology Department, Philippe Bastin from the Parasitology Department, Marc Lecuit and Michel Chignard from the Department of Infection and Epidemiology, Eliane Meurs from the Virology Department). These groups actively participate in seminars and in the annual departmental retreat.

The Cell Biology and Infection Department: Perspectives

The Department of Cell Biology and Infection which has never been evaluated as such since its creation in 2002 and at that time directed by PJ Sansonetti has reached a high international visibility as revealed by the recent high success of the international call for application for creation of G5 groups : one third of the 127 applications were from applicants interested to join our department and in addition, their quality was very high.

We thus want to continue our experience of such a successful department which associates units focusing on fundamental Cell Biology questions and units studying host-pathogen interactions, together with technological platforms.

Most initial interests of this department have remained intact but some have really shifted. For example, one of our goals is to continue to perform in depth analysis of cytoskeleton rearrangements upon infection or other events (migration, polarisation, ...) with a special

emphasis on actin, microtubules and other structures which affect intracellular organelles and trafficking but we do not want to stay any longer at a descriptive qualitative level. All of us are now convinced of the critical importance of quantitative image analysis. In this respect, several of us are strongly convinced that an interface with Physics and Mathematics for experimental modeling, -as well as for gene expression analysis- is increasingly required.

Several groups are increasingly interested in understanding in detail the impact of infection on the cell transcriptional program, and also on the chromatin structure and /or on the cell cycle. For these different aspects, as said above, the challenging issue is the study at the single cell level and the consequences at the tissue level.

For several of the microorganisms analyzed in the department, post-genomic studies will be pursued to unravel the function of factors interacting with the host or regulating the expression of the virulence factors.

Most groups now want to extend as much as possible their in vitro studies to the whole animal and for that, we wish to develop tissular microbiology as much as possible. This requires new instruments (e. g. two photon microscopes), new technologies and huge animal infrastructures that the new building should hopefully provide. Finally, recent extensive efforts to understand the host response has shifted the interest of some groups towards an increasing interest in the understanding of the normal situation, in particular at the host barriers. The role of commensals and probiotics is increasingly recognized as critical for the establishment of infections and will be tackled in the next years.

The department has a reasonable size, with a good balance between Microbiologists and Cell Biologists. Of course, we are well aware that many groups in the Institut Pasteur are using approaches similar to ours at some stage of their studies. We thus really continue to play a strong role in « promoting the in depth analysis of the cell biology/ microbiology-virology-parasitology interface ». However, the mammalian cell is highly complex and we believe that the stronger the Cell biology will be in our Department, the stronger the science will be at the Institut Pasteur.

Towards these goals, we are interested in reinforcing the department by bringing external new groups in the domains cited above, in particular groups working in trafficking, groups working on the various aspects of the nucleus (we lost a group working on this aspect) i.e. nuclear structure and dynamics, nucleo-cytoplasmic transport, chromatin remodeling, telomeres etc.

Finally, one of our strong wishes is to succeed to limit the geographical dispersion of the different groups of our department in the whole Pasteur campus.

The Cell Biology and Infection Department: Highlights

Highlights of recent years in alphabetic order by laboratory

Cossart Pascale. Bacteria-Cell Interactions Unit.

Our research focuses on the analysis of the infectious process by intracellular bacteria, using as a model system *Listeria monocytogenes* and to a lesser extent *Rickettsia*. It has led to

- The discovery and or characterization of several new virulence factors (FbpA, Auto, SOD, InlJ) and regulatory circuits (VirR, Stp) controlling virulence and to the discovery of non-coding RNAs
- The elucidation of the role of lipid rafts in the entry of *Listeria*
- The discovery of novel components allowing internalin-mediated entry and adherens junction formation
- The discovery of post-translational modifications of E-cadherin during internalin-mediated entry
- The identification of new factors essential for the InlB-dependent entry pathway
- The discovery of a new role for clathrin in bacterial invasion and adhesion
- The first report on a role for a septin in bacterial invasion
- The discovery of the first peptidoglycan modification allowing escape from the host immune system
- The discovery of histone modifications induced by LLO down regulating key immunity genes
- The demonstration that InlB as internalin has a species specificity and plays no role in the crossing of the intestinal barrier
- The epidemiological evidence that internalin is a virulence factor
- The establishment of the conjugated action of internalin and InlB in placental listeriosis
- The discovery of a novel actin nucleator produced by *Rickettsia conorii*
- The identification of the first *Rickettsia* receptor and its bacterial ligand

Dautry Alice. Biology of Cell Interactions Unit.

The unit focuses on two topics : 1) host-pathogen interactions in the infection by the obligate intracellular bacteria *Chlamydia* which are responsible for numerous pathologies(sexually transmitted diseases, respiratory tract infections); 2) the clathrin-independent internalization pathway used by cytokine receptors essential for lymphocyte development and proliferation. Our mains results are :

- The identification of several new bacterial proteins translocated into the host cell during infection,
- The demonstration that one of these proteins gets inserted into the membrane of the compartment in which the bacteria develop, mimics a fundamental motif for membrane fusion in eukaryotic cells, the SNARE motif and is essential for the recruitment of specific host SNARE proteins around the inclusion, providing the first example of SNARE mimicry by an intracellular bacterium.
- The demonstration that the internalization process of cytokine receptors does not involve clathrin nor caveolin, although, like the clathrin or caveolin-dependent pathways, it also uses the actin cytoskeleton.
- The identification of a cascade composed of Rac1, Paks and cortactin, which stimulates specifically the endocytosis of cytokine receptors, indicating that proteins involved in several entry pathways, such as cortactin, could be regulated differently depending on the pathway.

David-Watine Brigitte (Ulf Nehrbass). Nuclear Cell Biology Unit.

- **Functional architecture in the nucleus of *Saccharomyces cerevisiae*:**
- Initially working on nuclear structure function analysis, we have identified two proteins Mlp1 and Mlp2. In a series of projects we were able to show that these proteins structurally defines a nuclear envelope domain, relative to which genes can define functional states, and which harbours postranscriptional processing activity in a spatially

constrained fashion. To perform this analysis, we developed in collaboration with the LAIQ an application for automatic detection and localization of single fluorescently tagged loci in 3D+time.

- The gene localization method was extended by introducing a second nuclear landmark: the nucleolus. This implementation allowed the gene position information of aligned nuclei to be merged thus giving access for the first time to statistical robust high precision localization probability maps.
- **Functional analysis of nuclear envelope and NPC components in the nucleus of *C. elegans*: role at the cell and at the organism level (Group of V. Galy).**

Dejean Anne. Nuclear Organization and Oncogenesis Unit.

The work in our lab is dedicated to the study of the molecular and cellular mechanisms underlying the development of human cancers with a particular emphasis on the role of the post-translational modification by SUMO. Our work combines fundamental projects, based on experimental systems, as well as more clinically-oriented projects devoted to the study of human liver cancer.

- **PML in cellular senescence and gene expression**
We have clarified the mechanism of action of the PML tumor suppressor, a major SUMO substrate, in cellular senescence and have unravelled a new role for PML in higher-order chromatin organization and gene regulation through interaction with the nuclear matrix associated SATB1 protein.
- **Role of the SUMO pathway in vivo**
We generated mouse models deficient/attenuated for sumoylation and revealed a major role for the E2 Ubc9 enzyme, and hence sumoylation, in nuclear architecture and function, chromosome segregation and for embryonic viability in mammals. We provided the first evidence for a direct role of an E3 SUMO ligase, PIASy, in cellular senescence and apoptosis. We further identified PIASy as an important regulator of FIP200 function in TSC/mTOR signaling. We identified a new mechanism for regulation of PARP1 transcriptional activity through a sumoylation-coupled ubiquitination process. Finally, a new role of the SUMO pathway in telomere length maintenance was demonstrated in the fission yeast *S. pombe*.
- **Genetics and epigenetics of hepatocellular carcinoma**
We have shown that, in HCC, aflatoxin exposure plays a role in chromosome instability irrespective of the p53 status. Moreover, we defined a signature of 14 miRNAs specifically associated with liver cancer progression and identified miR221 as a potent oncogene.

Enninga Jost. Dynamics of Host-Pathogen Interactions Five-year group.

Our group "Dynamique des interactions hôte-pathogène" was created in January 2008. We are developing innovative microscopic approaches to investigate the cross-talk between invasive bacterial pathogens and the induced host responses on the molecular and cellular level. We have achieved and work on the following :

- Installation of our laboratory and recruitment of the team members to work on all proposed research projects.
- Establishing a microscopic assay that tracks the secretion of effectors from single bacteria in real time indicating the rapidity of this process during host cell entry
- Setting-up a robust and sensitive assay to track the time-point of vacuolar rupture by pathogens.
- Identification of bacterial factors that stabilize pathogen-containing vacuoles.
- Establishment of a novel approach to profile gene expression in single living cells.
- Determining the precise time-point of the pro-inflammatory gene induction during bacterial entry into host cells.

Etienne-Manneville Sandrine. Polarity and Cellular Migration Five-year group.

Since February 2006, when "Polarity and Cellular Migration G5" was initiated, we have focussed on three principal aspects of astrocyte polarity and migration:

- **Regulation of polarity and migration by the cellular environment.**
- We have shown that cadherin-mediated intercellular contacts provide polarity cues which promote asymmetric intracellular organization in immobile or migrating cells. Loss of N-cadherin in astrocyte-derived tumors leads to a loss of polarity and to random migration.
- **The intracellular signalling pathways controlling cell polarity.** We have studied the regulation and functions of key polarity proteins such as the small G protein Cdc42 and

the Par protein LKB1 and shown the involvement of evolutionary conserved polarity proteins such as Scrib (*Drosophila* Scribble) and AGS3 (*Drosophila* PINS). This study points to several tumor suppressors as key regulators of cell polarity and suggests that they may contribute this polarity function to their tumor suppressor role.

- **The organization, regulation and roles of the cytoskeleton.**

We have deciphered a new signalling pathway, involving Dlg1 and its binding partner GKAP, that promotes dynein interaction with microtubules. Such dynein regulation leads to microtubule anchoring at the leading edge plasma membrane and centrosome positioning and is likely to be involved in wide range of eukaryotic cell functions.

Guillén Nancy. Cell Biology of Parasitism Unit.

Our main interest is to obtain mechanistic insight into the cellular and molecular process responsible for human tissue invasion by the amoebic parasite *Entamoeba histolytica*, the agent of amoebiasis. In the last four years, we have:

- Participated in the NIH consortium that sequenced and published the amoebic genome.
- Set up the microarray technology that combined with proteomics strategies led us to identify new virulence factors including a family of lysine rich proteins involved in parasite invasion and in the onset of inflammation of the host tissue.
- Set up and validated the use of RNA interference for the molecular analysis of pathogenesis.
- Identified the human signalling pathways modulated at early stages of liver infection by the parasite and particularly, those responsible for cell death.
- Developed an *ex vivo* system of colon explants to study human intestine invasion by *E. histolytica* and evidenced the relevant impact of cysteine proteinases in the invasive process.
- Determined the physical parameters of parasite motility using novel imaging approaches.
- Discovered parasite chemotaxis towards tumour necrosis factor and revealed important roles for parasite adhesive molecules and the cytoskeleton during penetration of living tissue.
- Produced monoclonal antibodies suitable for diagnosis of *E. histolytica*.

Kellermann Odile. Stem cells, signalisation and prions Laboratory.

Focusing on the mechanisms that control stem cell differentiation and sustain the homeostasis of their differentiated progenies, we develop three major axes dealing with (i) the regulation of neuronal functions (ii) the role of the cellular prion protein and the impact of infection in a neuronal context (iii) the formation of mineralized tissue.

- We have exploited a neuroectodermal precursor able to convert into fully-functional serotonergic or noradrenergic neuronal cells to :
 - unravel control of 5-HT transport and antidepressant recognition by post-translational regulation of the serotonin transporter (SERT).
 - demonstrate that pathogenic prions impair monoaminergic functions through the production of bioamine-derived neurotoxins
 - identify CREB, immediate early genes and MMP9 as targets of PrP^C signaling.
- Using mesoblastic stem cells, we have identified the serotonergic receptor (5-HT_{2B}R) as a novel player in mineralization fully controlling the activity of the tissue-non specific alkaline phosphatase activity (TNAP).
- We have isolated murine dental pulp progenitor cell lines to set up repair protocols.
- We are developing new 3D X-ray scanner imaging technics to characterize phenotypic defects in genetically modified mice.

Israel Alain. Cell signaling and activation Unit (SMAC).

- **The NF- κ B signaling pathway :** we have demonstrated that IKK, the core kinase complex of the NF- κ B cascade, also plays a negative role by inducing the degradation of Bcl10, a critical component of the TcR signaling pathway. We have completed (in collaboration with M Véron/ F Agou) a detailed structure-function analysis of NEMO, the regulatory element of the IKK complex, and identified a unique bipartite ubiquitin-binding domain responsible for the ability of this protein to recognize K63 linked polyubiquitin chains.

- **The NRP/optineurin protein** : we have demonstrated that this NEMO-related Golgi-associated protein, in addition to its function in protein exocytosis, is a new component of the innate antiviral response.
- **The Notch pathway** : we have clarified the function of Itch, an ubiquitin-ligase known to negatively regulate Notch activation in *Drosophila*, and demonstrated that Itch is responsible for lysosomal degradation of non-activated Notch in mammalian cells, through the use of atypical K29-linked polyubiquitin chains. We have also demonstrated that AAK1, a kinase known to regulate clathrin-mediated endocytosis, is involved in the early steps of Notch activation and counteracts the activity of Numb. In parallel we have dissected the mechanisms responsible for generating active Notch ligands : genetic studies had suggested that ubiquitination and endocytosis of the ligands were required for activation of Notch signaling; our biochemical analysis has further demonstrated that the intracellular region of the ligands regulates their trafficking, and that an ubiquitination-dependent recycling step was required for their full signaling activity.

Olivo-Marin Jean-Christophe. Quantitative Image Analysis Unit.

We have developed several projects related to biological image analysis that address the following topics:

- Detection and tracking of microscopic spots in 3D+t image data: design methods able to detect with high accuracy multiple biological objects moving in three-dimensional space and follow moving spots switching between different dynamics characteristics; combine image model estimation and kinetic model estimation
- Image restoration and object detection in microscopy: achieve an improved detection of objects visualised in microscopy by taking into account the Point Spread Function (PSF) of the imaging system
- Microscopic image denoising: based on biorthogonal Haar-domain hypothesis tests or a multiscale variance stabilizing transform (MS-VST) for mixed Poisson-Gaussian noise process
- Detection of curvilinear objects: an automatic real time method based on the beamlet transform for the detection of curvilinear structures, and in particular below optical resolution filaments like or DNA filaments microtubules
- Cell shape and motility analysis: 3D robust region models and multiple level sets coupled by a non-overlap constrain
- Segmentation of colour images: applications in histopathology, cytology and screening; a non-parametric classification was developed to automatically separate aggregated cells.

Prévost Marie-Christine. Ultrastructural microscopy (Imagopole).

In the last three years we established several new technologies providing the right tools to address morphological questions at high resolution:

- High pressure freezing to avoid artifacts of conventional room temperature (RT) sample preparation techniques.
- Installation of the cryo-SEM, which allows the observation of the surface from samples, which are sensitive to RT-preparation techniques as biofilms.
- Introduction of Cryo Electron Microscopy Of Vitreos Sections (CEMOVIS) to analyze cellular structures in their native state at high resolution.
- Correlative Light Electron Microscopy (CLEM) that combines the dynamics of live cell imaging with the resolution of EM analysis.
- Setting up of (cryo)-electron tomography on the cryo-electron microscope thanks to the purchase of a new high resolution CCD camera.

Sansonetti Philippe. Microbial Molecular Pathogenesis Unit

This Unit uses *Shigella* as a tool to understand how invasive pathogens disrupt, invade, and cause the inflammatory destruction of the intestinal epithelium, and based on this, to develop vaccines. More recently, we initiated a *Klebsiella* project to understand the mechanisms of acute and chronic pulmonary infections, and a project studying how commensals and pathogens affect the homeostasis of the crypt-villous axis of the gut epithelium. Here are some highlights since last evaluation: Discovery of Nod proteins as intracellular sensors of bacteria via muropeptides.

- Demonstration of Ca⁺⁺/ATP dependent surface nanopodes that capture bacterial bodies.

- Demonstration that c-src, activated by IpaC, is essential to entry signalling, achieving extended actin polymerization via Cortactin.
- Identification of MxiE, a *Shigella* regulon that controls expression of the Osp and IpaH proteins, demonstration that these proteins are essentially regulating the innate response: IpaH proteins form a new family of E3 ligase; OspG is a kinase that binds the E2 transfer enzyme, acting as a potent anti-inflammatory molecule; OspF dephosphorylates MAPKinases, and causes epigenetic suppression of expression of key genes of the innate response, including IL-8.
- Demonstration that *Shigella* effectors under the MxiE regulon collectively suppress the expression of basic elements of epithelial defence such as antimicrobial peptides.
- Discovery of the oxygen-dependant competence of the type III secretion system of *Shigella*.
- Development of a murine model of rhinoscleroma, Mickulicz cells are "inflammatory monocytes".
- Demonstration of the anti-inflammatory role of *Lactobacillus casei* on epithelial cells.
- Development of a novel strategy of parenteral vaccination based on the synthesis of complex sugars subsequently conjugated to a carrier protein.
- Successful completion of a phase 1 and a phase 2 trials of a *Shigella dysenteriae* 1 live oral vaccine candidate, SC599.

Shorte Spencer. Dynamic Imaging (Imagopole).

We develop and apply optical imaging methods to the study of host-pathogen interactions at a molecular, cellular, tissue and whole organism level, notably using analysis of spatial and temporal dynamics in situ. We use fluorescence and bioluminescence based high-content imaging techniques in three main areas of interest: 1) development and application of new molecular probes; 2) development of novel imaging modalities, and/or biological models, and 3) development of image analysis, database & visualization solutions. Selected published works include:

- First in vivo visualization of Plasmodium transmission from mosquito to mammalian host.
- Quantitative 4D-visualization of HIV-1 viral-entry and dynamics within host cell
- Demonstrated novel use of image based photon-counting methods enabling real-time quantitative imaging of viral gene expression in infected single primary cells.
- Invented technique for super-resolution 3-D imaging of living cells in micro-fluidic flow suspension (micro-rotation and confocal axial tomography (awarded 2005 French Engineers of the Year prize).

Zimmer Christophe. Imaging and Modeling Five-year Group.

Our group develops computational imaging and modeling approaches to gain a quantitative understanding of cell biological processes. Our current work focuses on the spatial organization of the genome inside yeast nuclei and the development of super-resolution microscopy. Recent results:

- We have developed and validated a computational technique, which automatically analyses images of thousands of nuclei and generates high resolution maps of gene territories in the yeast *Saccharomyces cerevisiae*.
- We found that several genomic loci are confined to 'gene territories' much smaller than the nucleus, which can be remodeled during transcriptional activation.
- We found that coregulated genes do not necessarily occupy the same subnuclear compartments.
- We found that the intranuclear position of many genomic loci, including most telomeres, are strongly determined by the genomic distance from the centromere.

Zurzolo Chiara. Membrane Traffic and Pathogenesis Unit.

Chiara Zurzolo moved to our Department in 2003. Her principal projects are:

- 1) Understanding the mechanism of GPI-anchored protein (GPI-AP) sorting to the plasma membrane (PM) of polarized epithelial cells
- 2) Study of the intracellular trafficking of the prion protein, the site of its pathological conversion, and the mechanisms of intercellular spreading of the infection

By using a combined approach of biochemistry and imaging in live cells they have found that:

- GPI-APs are directly sorted to the apical domain of polarized living cells
- Different Snares are used for the direct and indirect apical pathway
- Rafts are not sufficient for sorting of GPI-APs, but oligomerization is required
- Protein oligomerization is dependent on both the ectodomain, the GPI anchor and/or the

- lipid environment both at the plasma membrane and in the Golgi apparatus
- GPI-APs are organized in homo clusters independent of cholesterol that can form cholesterol dependent hetero clusters
- Raft-association of PrP^C is necessary for its correct folding in the ER
- Interaction between wild-type and mutant PrP forms might occur in rafts, but the raft environment may protect PrP mutants from conversion
- The proteasome is not involved in the degradation of PrP mutants
- Both raft-dependent and clathrin-dependent pathways are involved in PrP endocytosis
- PrP pathological conversion occurs in the endosomal recycling compartment
- Spreading of infectious prions between neuronal cells and between dendritic cells and neurons occurs through Tunneling Nanotubes.

March 1, 2009

CELL BIOLOGY AND INFECTION DEPARTMENT	Permanent Scientists (including the Head of Units)				Post-Doc	Students		Engineers	Technicians	TOTAL
UNITS	IP	CNRS	INSERM	OTHERS		Master	Thesis			
Bacteria-Cell Interactions Pascale Cossart	4	—	—	2	7	—	4	3	1	21
Biology of Cell Interactions Alice Dautry	2	1	—	—	1	1	3	—	3	11
Cell Biology of Parasitism Nancy Guillén	2	1	—	1	2	1	3	1	1	12
Cell signaling and activation Alain Israël	3	3	—	—	1	1	5	1	3	17
Membrane Traffic and Pathogenesis Chiara Zurzolo	1	—	—	—	4	—	4	1	1	11
Molecular Pathogenesis Philippe Sansonetti	2	—	2	1	8	—	5	2	4	24
Nuclear Organisation and Oncogenesis Anne Dejean	3	1	1	—	4	—	2	1	1	
Quantitative Image Analysis Jean-Christophe Olivo-Marin	1	—	—	—	2	3	2	2	—	10
FIVE-YEAR GROUPS										
Cell Polarity and Migration Sandrine Etienne-Manneville	—	1	1	—	2	—	2	1	—	7
Dynamics of Host-Pathogen Interactions Jost Enninga Since January 1, 2008	1	—	—	—	2	1	1	—	1	6
Imaging and Modeling Christophe Zimmer Since January 1, 2008	1	—	—	—	2	—	1	1	—	5
PLATFORMS										
Dynamic Imaging Spencer Shorte	—	1	—	—	2	—	—	5	—	8
Ultrastructural Microscopy Marie-Christine Prevost	—	—	—	—	—	1	—	5	4	10
ASSOCIATED LABORATORY										
Stem cells, signaling and prions Odile Kellermann	1	1	2	1	2	1	4	—	2	14
TOTAL STAFF OF THE DEPARTMENT	21	7	6	5	39	9	36	23	21	156